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SHORT COMMUNICATION

GABA and the ornithine δ-aminotransferase gene in vigabatrin-associated visual field defects

FUKI M. HISAMA, RICHARD H. MATTSON, HELEN H. LEE, KRISTIN FELICE & OGNEN A. C. PETROFF

Department of Neurology, Yale University School of Medicine, New Haven, CT, USA

Correspondence to: Fuki M. Hisama, Department of Neurology, P.O. Box 208018, 333 Cedar St., New Haven, CT, USA. *E-mail*: fuki.hisama@yale.edu

Vigabatrin use in some epilepsy patients has been associated with persistent visual field constriction and retinal dysfunction. The mechanism is unknown, but could be related to vigabatrin, chronic epilepsy, GABA toxicity, or the effect of a metabolite in combination with a predisposing genotype. The aim of this study was to investigate the latter two hypotheses. Levels of brain gamma-aminobutyric acid (GABA) measured by nuclear magnetic resonance spectroscopy were similar in subjects taking vigabatrin who developed visual field constriction and those who did not. We tested whether allelic heterogeneity of the ornithine aminotransferase gene occurs in the affected patients. No clinically significant mutation was detected, although a common intronic polymorphism was identified.

Key words: vigabatrin; GABA; visual field constriction; epilepsy.

INTRODUCTION

Vigabatrin is a relatively selective, irreversible inhibitor of GABA-transaminase, resulting in increased amounts of brain gamma- aminobutyric acid (GABA) and ornithine[1]. Vigabatrin is used in over 60 countries in the treatment of partial epilepsy and infantile spasms. Persistent, sometimes severe visual field constriction was considered a rare (< 1:1000) occurrence, until reports in 1997–1999 found visual field constriction in as much as 40% of subjects on vigabatrin monotherapy[2–4]. Follow-up of these patients 3–6 months after stopping the drug has shown largely no improvement in the visual field defect[5]. The cause of the visual field constriction is unknown. Ocular electrophysiologic studies have shown abnormal retinal responses, the likely site of damage[6–9]. Several mechanisms have been postulated, including toxicity of GABA, a consequence of epilepsy, antiepileptic drug side effect, vigabatrin alone or in combination with other antiepileptic drugs, and finally, genetic variation in the mitochondrial enzyme ornithine δ-aminotransferase (OAT)[10,11]. In the autosomal recessive condition, gyrate atrophy of the choroid and retina mutations in the OAT gene cause progressive peripheral and central visual field loss beginning in the second decade[12,13]. We report here results of proton magnetic resonance spectroscopic measurements of GABA in 17 adult patients (aged 25–71 years, eight men, nine women), taking vigabatrin for refractory complex partial epilepsy, and molecular analysis of the OAT gene in three subjects with vigabatrin-associated visual field defect determined by Goldmann perimetry.

MATERIALS AND METHODS

Genomic DNA was extracted from venous blood samples obtained under an ethically approved protocol. Individual exons of the OAT gene were amplified by PCR using previously published primer sets[14]. In ad-

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dition, the following primers were used to amplify Exons 7 and 11: 7F: 5′ TAT GCT TTC AGA TTT CCA AGT G 3′; 7R: 5′ CAT CAC AAA CAG CTA ACT CGA C 3′; 11AF: 5′ CAT ACA TAT GGC AAG GGA TGT 3′; 11AR: 5′ TAG AGG ACT TGA TTT AGA GGC 3′; 11BF: 5′ AGA ACA ACG TTT ATG AAC CTG 3′; 11BR: 5′ TCA TCA CAA AAC AGA CAT TTG 3′. The reaction mixture (50 μL) contained 100 ng genomic DNA, 10 mmol Tris-HCl (pH 8.3), 60 mmol KCl, 100 ng specific primers, $MgCl_2$ of 1.5 mmol (Exons 7, 11), 2.0 mmol (Exons 3, 4, 8, 9), 3 mmol (Exons 5, 10), 4 mmol (Exon 6), and 200 μmol dNTPs, 2.5 U Taq DNA Polymerase (Perkin-Elmer). PCR products were electrophoresed on 1% agarose gels and visualized with ethidium bromide. After purification using QiaQuick columns (Qiagen), PCR products were sequenced on an Applied Biosystems automated DNA sequencer model 373A. Mutations were confirmed by sequencing both strands, and amplification followed by sequencing of a second PCR product.

Proton magnetic resonance spectroscopy was performed at 2.1 Tesla (89.43 MHz for ^{1}H) with an 8 cm distributed capacitance ^{1}H transceiver radio-frequency coil. From the scout image, a 3.0 × 1.5 × 3.0 cm (14 cm^3) volume in the occipital cortex was chosen for spectroscopic measurements. Homonuclear editing of the 3.0 ppm C4 GABA resonance was performed using the spin–spin (J) editing pulse sequence described previously[15]. The localization techniques were 3D-ISIS sequence, outer volume suppression, plane selective excitation, and a surface spoiler coil. An inversion recovery pulse and a semiselective refocusing pulse were used for water suppression. Spectral editing was used to separate the GABA C4 resonance at 3.0 ppm from overlapping resonances by applying a 26.5 ms DANTE pulse applied symmetrically in time about the center of the sequence to improve editing selectivity to the 1.9 ppm C3 resonance. Spectral acquisition conditions were TR 4.1 s, TE 68 ms, sweep width 15000 Hz, and acquisition time 546 ms.

RESULTS

Direct DNA sequencing of the entire coding region of the OAT gene in three subjects with vigabatrin-associated visual field defect detected no functional mutations. The only sequence variant IVS6 + 14 G → A, found in one of the three affected subjects, has been previously reported as a common polymorphism with a worldwide distribution[14]. Median-edited GABA (GABA plus homocarnosine) was the same in affected (2.5 mM, number 3, range 2.3–3.5) and unaffected (2.4 mM, number 14, range 2.0–2.9, interquartile 2.3–2.8) patients. The vigabatrin doses were similar for the affected (median 5 g per day, number 3, range 4.5–6) and unaffected (median 4, number 14, range 2–6, interquartile 3.1–4.5) patients.

DISCUSSION

The mechanism of vigabatrin-associated visual field defect is unknown. The relationship between dose or duration of therapy with vigabatrin and the risk of developing visual constriction is controversial. Although vigabatrin weakly binds to a variety of transaminases, its direct effect on OAT is thought to be minimal. The increase in ornithine is attributed to the increase in GABA inhibiting OAT[1]. Roubertie[11] proposed a partial defect in OAT made complete by vigabatrin as the mechanism for retinal toxicity because of similarities between the vigabatrin-associated visual field defect and gyrate atrophy of the choroid and retina. Although, theoretically, subjects that are heterozygous carriers or have an otherwise benign polymorphism in the OAT gene could be at higher risk of an adverse drug effect, the absence of mutations in three affected subjects suggests that it is not required for the vigabatrin-associated field defect to occur. In addition, Krauss *et al.*[11] have reported normal serum ornithine levels in two vigabatrin-treated subjects with visual field loss.

Another proposed mechanism is direct retinal toxicity by above normal GABA concentrations. If GABA were the toxic agent, then GABA levels might predict the risk of developing the visual abnormality. We found that brain GABA levels were not significantly higher in vigabatrin-treated patients who experienced visual field constriction, compared with those who did not. Another point against the GABA hypothesis is that there are several other antiepileptic drugs, such as gabapentin, lamotrigine, tiagabine, topiramate, that increase GABA in the human visual cortex[15–17], but similar retinal complications have not been reported with their use[5].

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